Hepatoprotective effects of systemic ER activation

BulkRNAseq - Transcriptome molecular signatures

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```
# source and library import
source('code/00_helper_functions.R')
library(tidyverse)
library(DESeq2)
library(VennDiagram)
library(clusterProfiler)
library(org.Mm.eg.db)
library(ggrepel)
```

```
# color palettes
colPals <- list()
colPals$conditions <- setNames(c('#E98BB6', '#B02262', '#7F9AD7', '#2A2F72', '#7DC7D1', '#339ACD', '#350
                                 c('CDf', 'HFDf', 'CDm', 'HFDm', 'DPN', 'DIP', 'E2', 'PPT'))
colPals$RdBu <- rev(RColorBrewer::brewer.pal(n=11, name = 'RdBu'))
colPals$UpDown <- setNames(colPals$RdBu[c(10,2)],
                           c('up', 'down'))
```

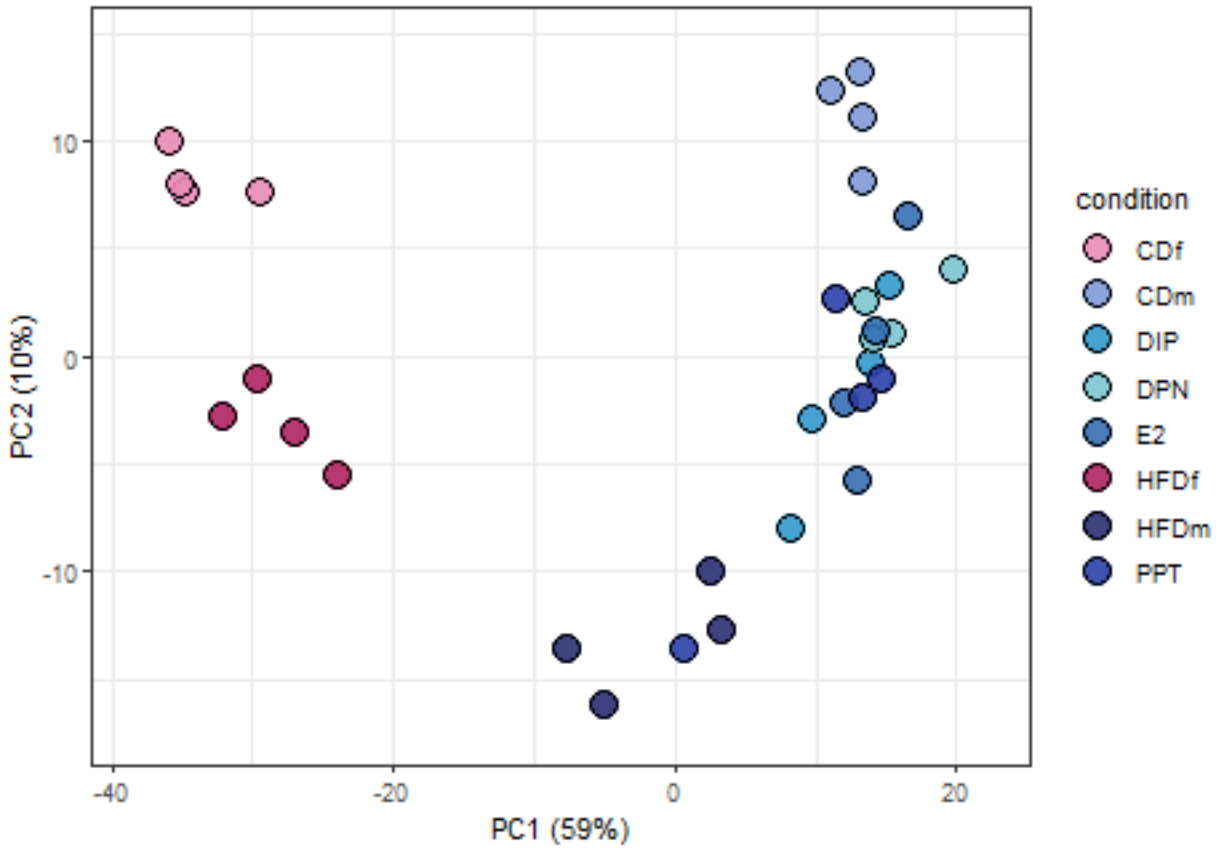
Load data

```
# consensus differentially expressed genes
DEGs <- readRDS('results/bulkRNAseq_mmus_DEGs.rds')

# raw counts RNAseq
raw_counts <- read.table(
  file = 'data/bulkRNAseq_mmus_rawcounts.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE) %>%
  dplyr::filter(geneID %in% DEGs$unfilt$CDfVsCDm$ensembl_gene_id) %>%
  tibble::column_to_rownames('geneID') %>%
  as.matrix()

# gene lengths
gene_len <- read.table(
  file = 'data/bulkRNAseq_mmus_gene_lengths.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE) %>%
  dplyr::filter(geneID %in% DEGs$unfilt$CDfVsCDm$ensembl_gene_id)
```

```r
# design RNAseq
design_meta <- read.table(
  file = 'data/bulkRNAseq_mmus_design.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE)

# ensembl gene annotation (Mus musculus)
gene_ann <- read.table(
  file = 'data/ensembl_mmus_sep2019_annotation.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE,
  fill = FALSE,
  quote = '') %>%
  dplyr::filter(ensembl_gene_id %in% DEGs$unfilt$CDfVsCDm$ensembl_gene_id) %>%
  dplyr::arrange(factor(ensembl_gene_id, levels = rownames(raw_counts)))
```

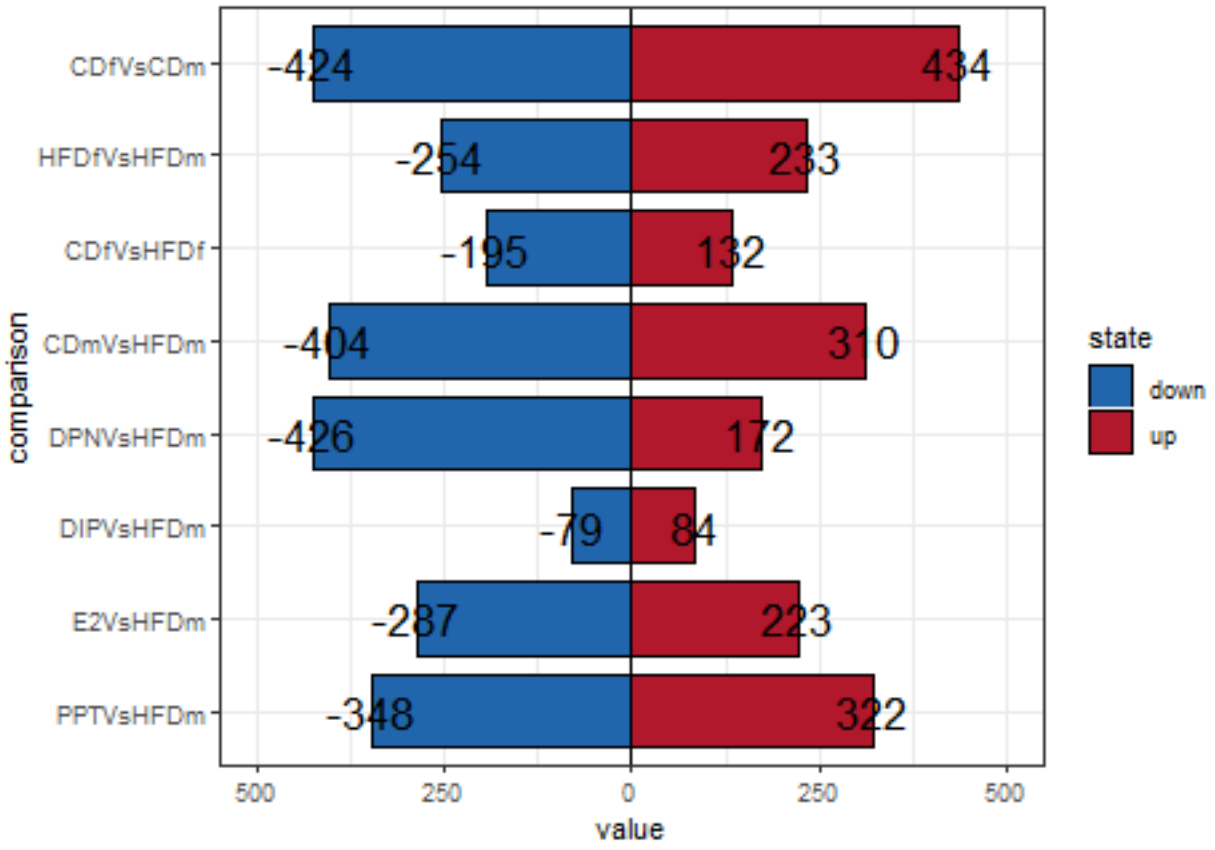
Principal component analysis (PCA)

```r
pca_res <- DESeq2::DESeqDataSetFromMatrix(countData = raw_counts,
                                  colData = design_meta,
                                  design = ~0 + condition) %>%
  DESeq2::estimateSizeFactors() %>%
  DESeq2::DESeq() %>%
  DESeq2::vst(blind = FALSE) %>%
  assay() %>%
  doPCA()

df <- data.frame(PC1 = pca_res$pcs$PC1,
                 PC2 = pca_res$pcs$PC2,
                 condition = design_meta$condition)

ggplot(df, aes(x=PC1, y=PC2, fill=condition),) +
  geom_point(shape=21, size=5, stroke=0.5, color='black') +
  scale_fill_manual(values = alpha(colPals$conditions, 0.9)) +
  scale_x_continuous(expand = expansion(mult = c(.1, .1))) +
  scale_y_continuous(
    expand =expansion(mult = c(.1, .1))) +
  xlab(paste0('PC1 (', round(pca_res$perc_var[1]), '%)')) +
  ylab(paste0('PC2 (', round(pca_res$perc_var[2]), '%)')) +
  theme_bw()
```



Differentially expressed genes (DEGs)

```
up <- lapply(DEGs$filt, function(x) sum(x$log2FoldChange>0)) %>% unlist()
down <- lapply(DEGs$filt, function(x) sum(x$log2FoldChange<0)) %>% unlist()

df <- data.frame(comparison=factor(rep(names(DEGs$filt),2), levels=names(DEGs$filt)),
                 state=c(rep('up', length(up)), rep('down', length(down))),
                 value=c(up, down*-1))

ggplot(df, aes(x=comparison, y=value, fill=state, label=value)) +
  geom_hline(yintercept = 0, linetype='solid', size=0.5) +
  geom_bar(color='black', size=0.5, width=0.8, position='stack', stat='identity') +
  geom_text(size=6) +
  scale_fill_manual(values = colPals$UpDown) +
  scale_x_discrete(limits = rev) +
  scale_y_continuous(limits = c(-500,500), labels = c(500,250,0,250,500)) +
  coord_flip() +
  theme_bw()
```

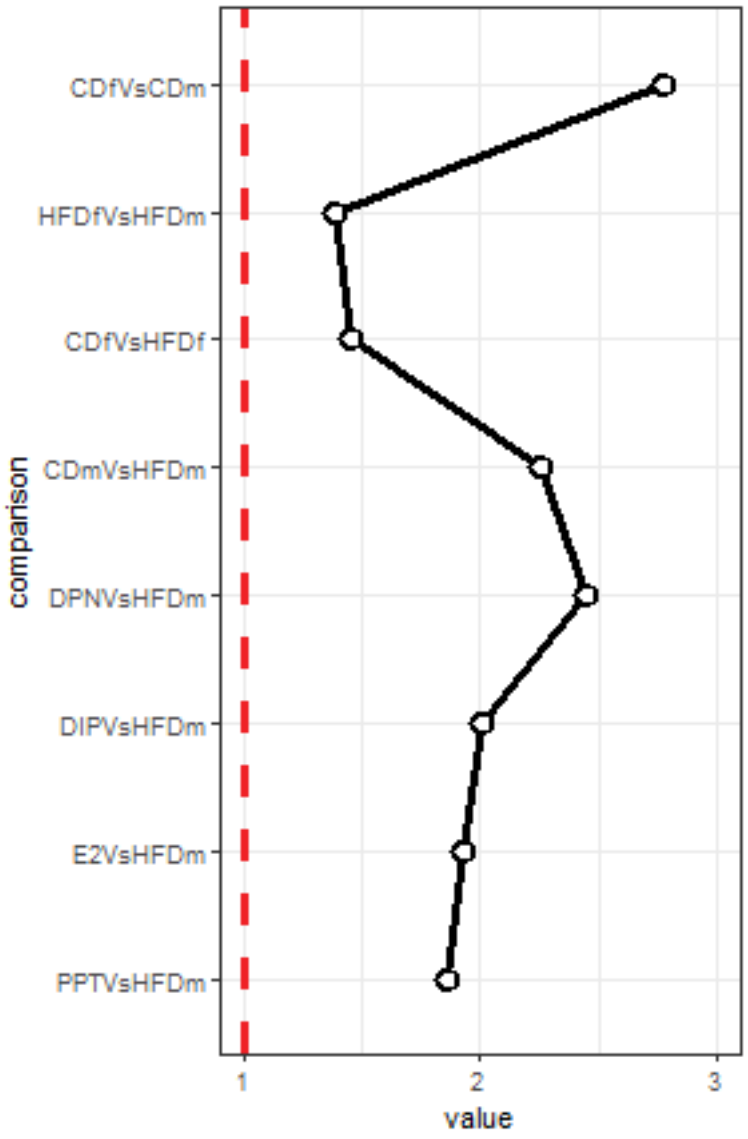


Filter and normalize RNAseq data

```
RNAseq <- list()

# remove outlier sample PPT_HFD_male_4
RNAseq$counts <- raw_counts %>%
  as.data.frame() %>%
  dplyr::select(-PPT_HFD_male_4)

RNAseq$design_meta <- design_meta %>%
  dplyr::filter(sample != 'PPT_HFD_male_4')

RNAseq$annotation <- gene_ann %>%
  dplyr::rename(geneID = ensembl_gene_id) %>%
  dplyr::left_join(gene_len, by = 'geneID')

RNAseq$cpm <- RNAseq$counts %>%
  normalizeData(method = 'CPM')

RNAseq$tpm <- RNAseq$counts %>%
  normalizeData(len = RNAseq$annotation$length, method = 'TPM')
```

Transcriptome-wide signal-to-noise ratios (tSNR)

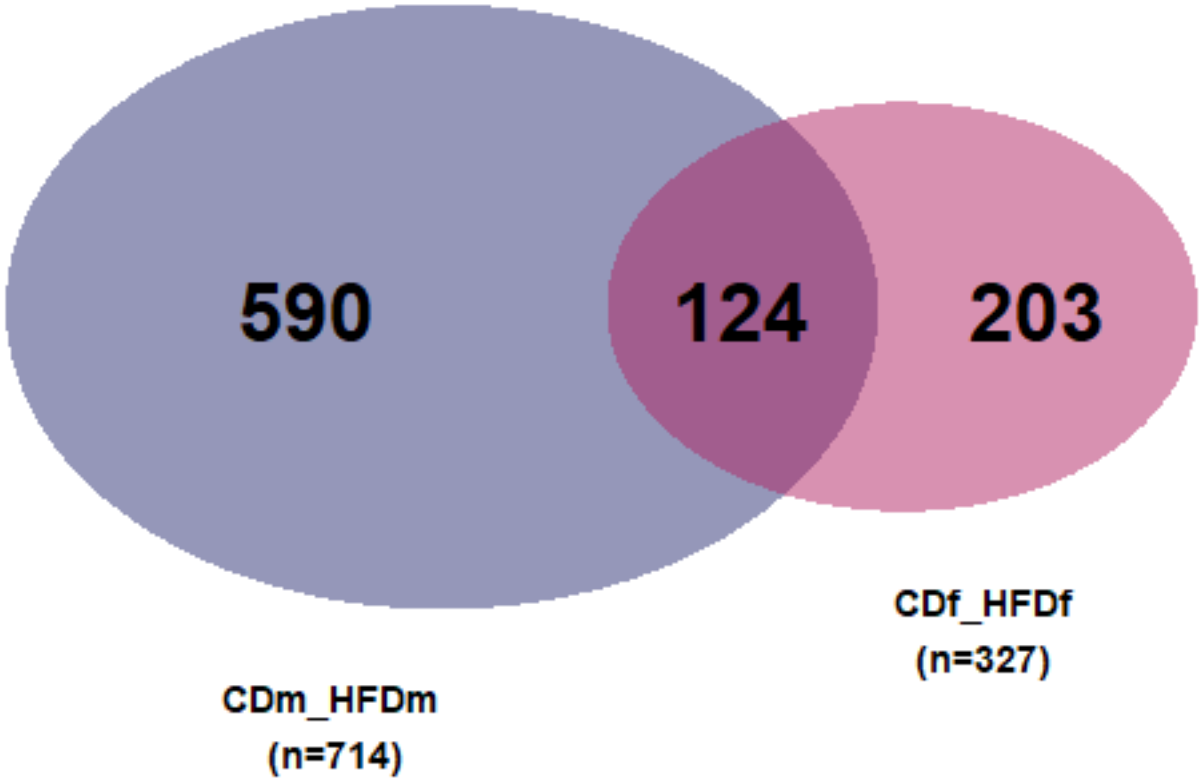
```
df <- RNAseq$tpm %>%
  scaleData(method = 'zscore') %>%
  tSNR(group.lbls = RNAseq$design_meta$condition) %>%
  tibble::rownames_to_column(var = 'X') %>%
  tidyr::pivot_longer(cols = dplyr::everything()[-1], names_to = 'Y') %>%
  tidyr::unite(col = 'comparison', X, Y, sep = 'Vs') %>%
  dplyr::filter(comparison %in% names(DEGs$filt)) %>%
  dplyr::mutate(comparison=factor(comparison, levels = names(DEGs$filt)))

ggplot(df, aes(x=comparison, y=value)) +
  geom_line(group=1, size=1.2) +
  geom_point(shape=21, size=3, stroke=1.5, color='black', fill='white') +
  geom_hline(yintercept = 1, linetype='dashed', size=1.2, color='#EF2126') +
  scale_x_discrete(limits = rev) +
  scale_y_continuous(limits = c(1,3), breaks = c(1,2,3)) +
  coord_flip() +
  theme_bw()
```



Plot Venn Diagram (Fig S1E)

```
CDf_HFDf <- DEGs$filt$CDfVsHFDf
CDm_HFDm <- DEGs$filt$CDmVsHFDm

grid.newpage()
myCol <- c("#B02262", "#2A2E72")
venn.plot <- venn.diagram(
  x = list(CDf_HFDf$ensembl_gene_id, CDm_HFDm$ensembl_gene_id),
  category.names = c("CDf_HFDf\n(n=327)", "CDm_HFDm \n(n=714)"),
  filename = NULL,
  output=T,
  lwd = 2,
  lty = 'blank',
  fill = myCol,
```

```
    cex = 2.5,
        fontface = "bold",
        fontfamily = "sans",
  cat.cex = 1.2,
        cat.fontface = "bold",
        cat.default.pos = "outer",
        cat.fontfamily = "sans",
  cat.pos = c(-10, 10),
        cat.dist = c(0.08, 0.08))

grid.draw(venn.plot)
```

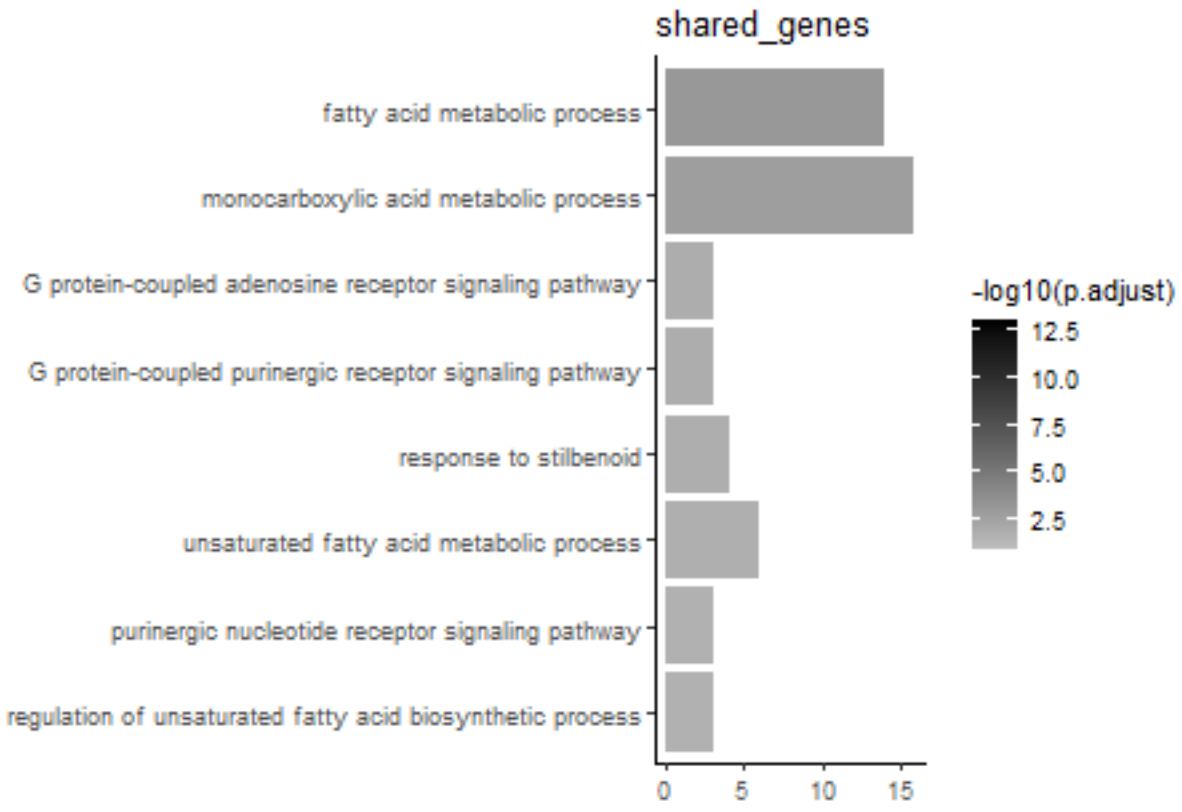


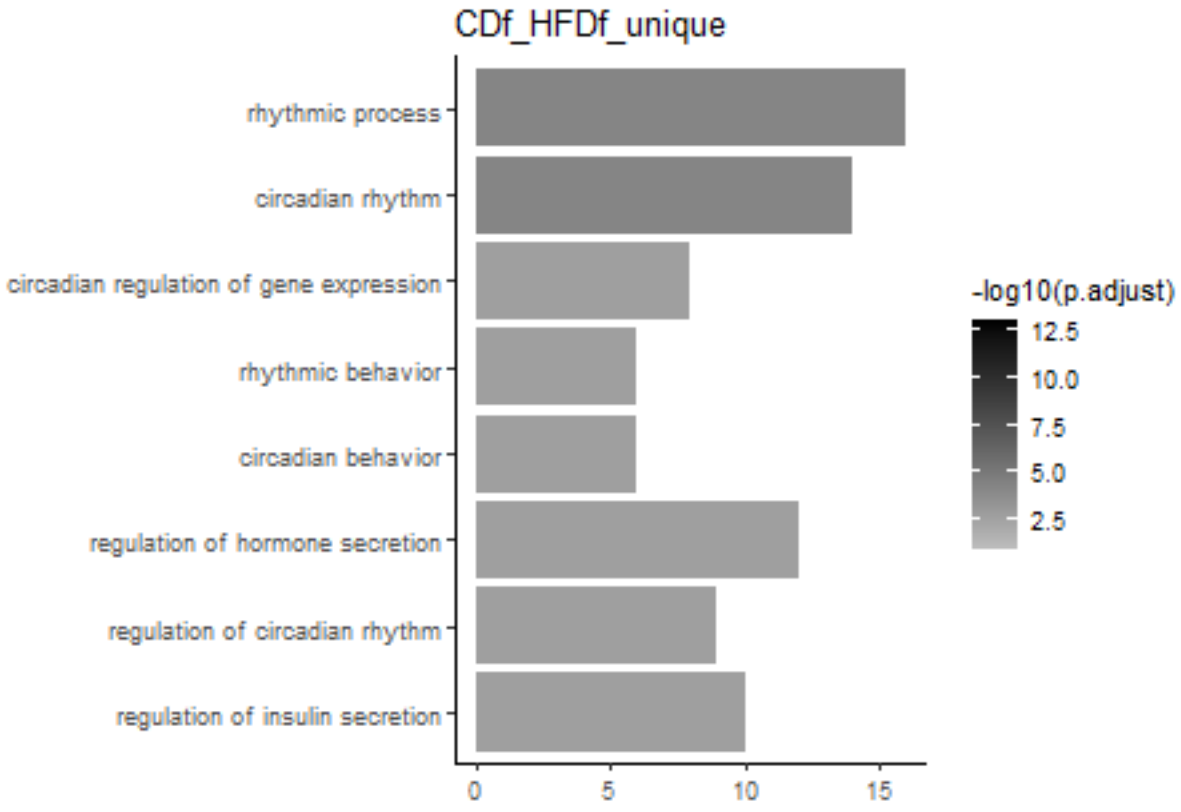

```
# Make intersection to extract the genes names from Venn.
intersections <- list("", "", "")
names(intersections) <- c("shared_genes", "CDf_HFDf_unique", "CDm_HFDm_unique")

intersections[[1]] <- intersect(CDf_HFDf$ensembl_gene_id,CDm_HFDm$ensembl_gene_id)
intersections[[2]] <- setdiff(CDf_HFDf$ensembl_gene_id, intersections$shared_genes)
intersections[[3]] <- setdiff(CDm_HFDm$ensembl_gene_id, intersections$shared_genes)
```

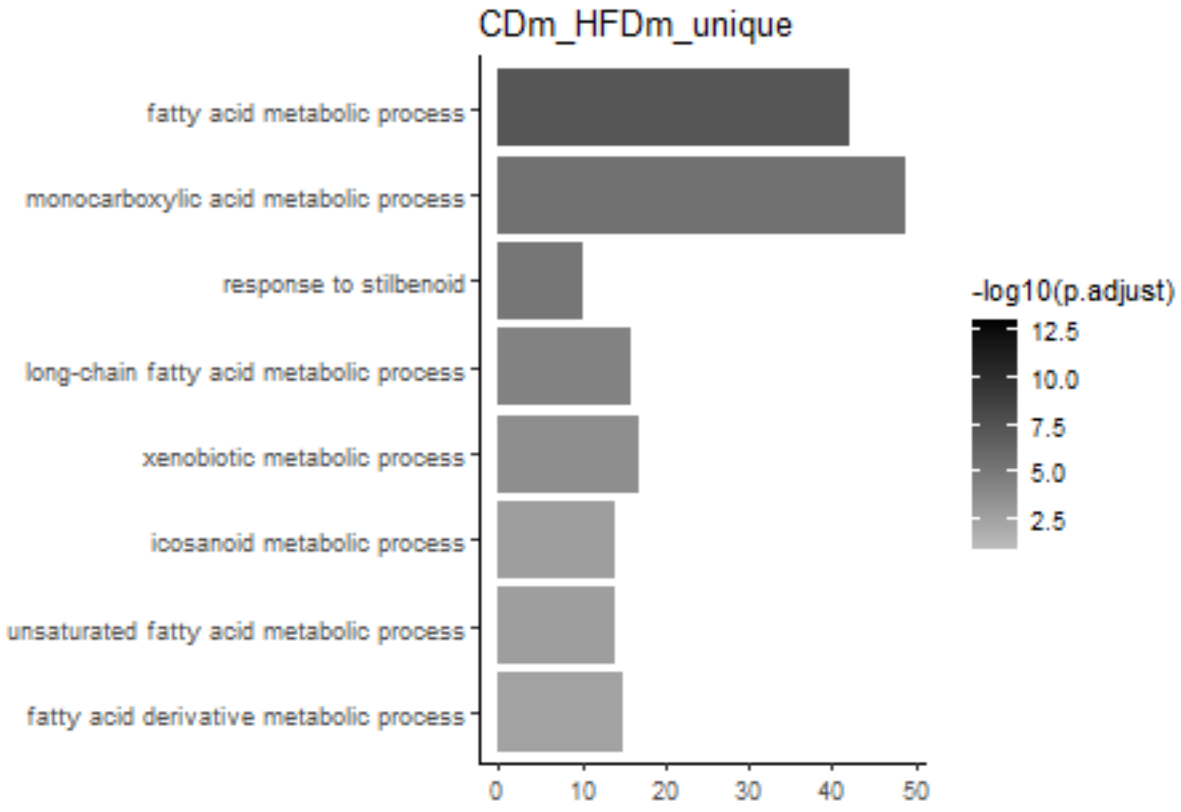
Gene Ontologies (Fig S1F,G,H)

```
background <- DEGs$unfilt$CDfVsCDm$ensembl_gene_id
options(connectionObserver = NULL) # workaround due to a bug
```

```
GO_list <- list(GO.results = list(),
                GO.top8 = list(),
                term.order.plotting = list())

for (i in 1:3) {
GO_list$GO.results[[i]] <- enrichGO(gene = intersections[[i]],
                keyType       = 'ENSEMBL',
                OrgDb         = org.Mm.eg.db,
                ont           = "BP",
                pAdjustMethod = "BH",
                pvalueCutoff  = 0.05,
                qvalueCutoff  = 0.05,
                minGSSize     = 3,
                readable      = TRUE,
                universe = background)
head(GO_list$GO.results[[i]])

name.me <- c("shared_genes", "CDf_HFDf_unique", "CDm_HFDm_unique")

GO_list$GO.top8[[i]] <- GO_list$GO.results[[i]]@result %>% filter(p.adjust<0.05) %>% mutate(GeneSet = na

GO_list$term.order.plotting[[i]] <- GO_list$GO.top8[[i]] %>% dplyr::pull("Description")

print(ggplot(GO_list$GO.top8[[i]], aes(x=Count, y=factor(Description, levels=rev(GO_list$term.order.plo
  geom_col(aes(fill=-log10(p.adjust))) +
  theme_classic() +
  xlab("") +
  ylab("") +
  ggtitle(paste(unique(GO_list$GO.top8[[i]]$GeneSet))) +
  scale_fill_gradient(low="grey", high= "black", limits=c(1,13)))

}
```


shared_genes
fatty acid metabolic process
monocarboxylic acid metabolic process
G protein-coupled adenosine receptor signaling pathway
G protein-coupled purinergic receptor signaling pathway
response to stilbenoid
unsaturated fatty acid metabolic process
purinergic nucleotide receptor signaling pathway
regulation of unsaturated fatty acid biosynthetic process
0
5
10
15
-log10(p.adjust)
12.5
10.0
7.5
5.0
2.5


CDf_HFDf_unique
rhythmic process
circadian rhythm
circadian regulation of gene expression
rhythmic behavior
circadian behavior
regulation of hormone secretion
regulation of circadian rhythm
regulation of insulin secretion
0
5
10
15
-log10(p.adjust)
12.5
10.0
7.5
5.0
2.5



Plot volcanos (Fig S1I,J)

```
# Load dataframes from rds object.
## DEG in CD comparison
CDf_CDm <- DEGs$filt$CDfVsCDm

## All genes in given comparisons with fold-changes
CDf_CDm_unfilt <- DEGs$unfilt$CDfVsCDm
HFDf_HFDm_unfilt <- DEGs$unfilt$HFDfVsHFDm

# CD comparison
## Maximum significance and fold-change are capped here.
CDf_CDm$padj[CDf_CDm$padj < 10e-30] <- 10e-30
CDf_CDm$log2FoldChange[CDf_CDm$log2FoldChange < (-8)] <- (-8)
CDf_CDm$log2FoldChange[CDf_CDm$log2FoldChange > (8)] <- (8)

CDf_CDm_unfilt$padj[CDf_CDm_unfilt$padj < 10e-30] <- 10e-30
CDf_CDm_unfilt$log2FoldChange[CDf_CDm_unfilt$log2FoldChange < (-8)] <- (-8)
CDf_CDm_unfilt$log2FoldChange[CDf_CDm_unfilt$log2FoldChange  > (8)] <- (8)

# HFD comparison
## We do not need the HFD DEG, because we will plot the CDf vs CDm DEG with the HFD fold-changes

HFDf_HFDm_unfilt$padj[HFDf_HFDm_unfilt$padj < 10e-30] <- 10e-30
HFDf_HFDm_unfilt$log2FoldChange[HFDf_HFDm_unfilt$log2FoldChange < (-8)] <- (-8)
```

```
HFDf_HFDm_unfilt$log2FoldChange[HFDf_HFDm_unfilt$log2FoldChange  > (8)] <- (8)

# Create up and downregulated genes in respective comparisons (larger/smaller than 0 is sufficient beca
male_biased_CD <- filter(CDf_CDm, log2FoldChange < 0)
nrow(male_biased_CD)
```

```
## [1] 424
```

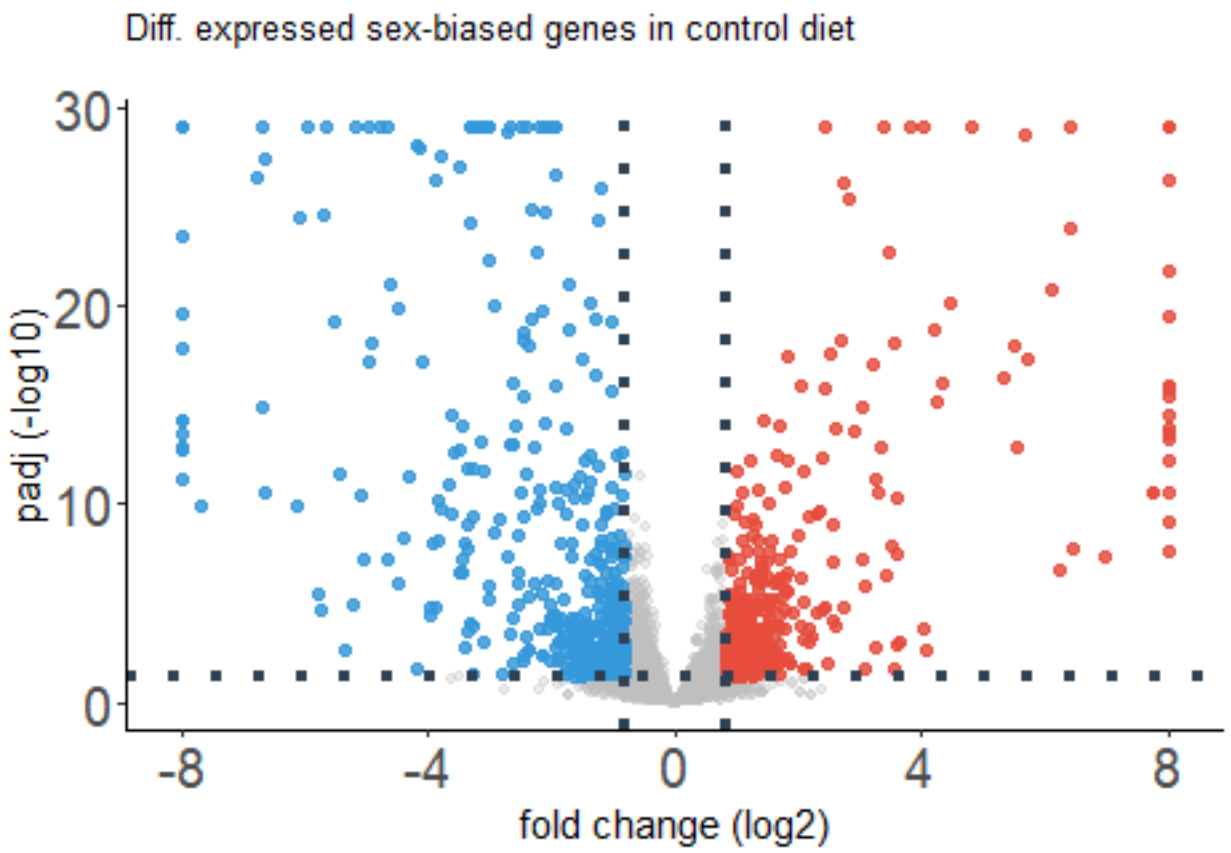
```
female_biased_CD <- filter(CDf_CDm, log2FoldChange > 0)
nrow(female_biased_CD)
```

```
## [1] 434
```

```
# These genes will be plotted in both, HFD and CD volcano plots, as sex-biased gene set.
male_biased_CD_vector <- filter(CDf_CDm, log2FoldChange < 0) %>% dplyr::pull("external_gene_name")
female_biased_CD_vector <- filter(CDf_CDm, log2FoldChange > 0) %>% dplyr::pull("external_gene_name")
# Filter the HFD table for genes that are sex-biased in CD.
male_biased_CD_in_HFDbckgrd <- HFDf_HFDm_unfilt %>% filter(external_gene_name%in%male_biased_CD_vector)
female_biased_CD_in_HFDbckgrd <- HFDf_HFDm_unfilt %>% filter(external_gene_name%in%female_biased_CD_vec
```

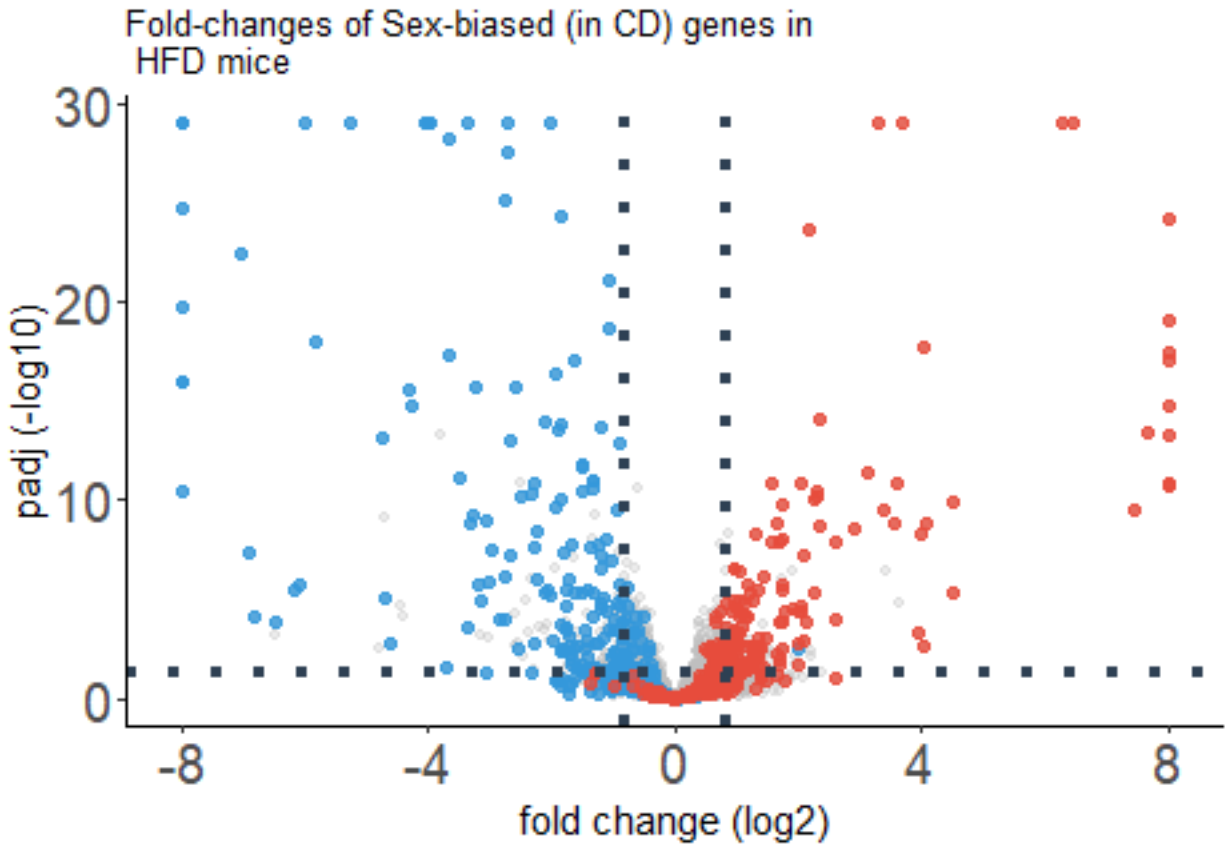
```
ggplot(CDf_CDm_unfilt) +
  geom_point(data = CDf_CDm_unfilt,
    aes(x = log2FoldChange, y = -log10(padj)),
    color = "grey",
    alpha = 0.3,
    cex = 1.5) +
  geom_point(data = male_biased_CD,
    aes(x = log2FoldChange, y = -log10(padj)),
    color = "#3498db", # #3498db is blue
    alpha = 0.8,
    cex = 2) +
  geom_point(data = female_biased_CD,
    aes(x = log2FoldChange, y = -log10(padj)),
    color =  "#e74c3c" ,
    alpha = 0.8,
    cex = 2) +
  theme_classic() +
  theme(axis.text = element_text(size=20),
        axis.title.x = element_text(size=15),
        axis.title.y = element_text(size=15)) +
  scale_x_continuous(limits = c(-8.1, 8.1), breaks =c(-8, -4, 0, 4, 8)) +
  xlab("fold change (log2)") +
  ylab("padj (-log10)") +
  geom_vline(xintercept = 0.807,
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
  geom_vline(xintercept = -0.807,
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
  geom_hline(yintercept = -log10(0.05),
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
```

```
  ggtitle("Diff. expressed sex-biased genes in control diet\n")
```




```
ggplot(HFDf_HFDm_unfilt) +
  geom_point(data = HFDf_HFDm_unfilt,
    aes(x = log2FoldChange, y = -log10(padj)),
    color = "grey",
    alpha = 0.3,
    cex = 1.5) +
  geom_point(data = male_biased_CD_in_HFDbckgrd,
    aes(x = log2FoldChange, y = -log10(padj)),
    color = "#3498db",
    alpha = 0.8,
    cex = 2) +
  geom_point(data = female_biased_CD_in_HFDbckgrd,
    aes(x = log2FoldChange, y = -log10(padj)),
    color =  "#e74c3c" ,
    alpha = 0.8,
    cex = 2) +
  theme_classic() +
  theme(axis.text = element_text(size=20),
        axis.title.x = element_text(size=15),
        axis.title.y = element_text(size=15)) +
  xlab("fold change (log2)") +
  ylab("padj (-log10)") +
  scale_x_continuous(limits = c(-8.1, 8.1), breaks =c(-8, -4, 0, 4, 8)) +
  geom_vline(xintercept = 0.807,
```

```
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
  geom_vline(xintercept = -0.807,
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
  geom_hline(yintercept = -log10(0.05),
    col = "#2e4053",
    linetype = "dotted",
    size = 1.5) +
  ggtitle("Fold-changes of Sex-biased (in CD) genes in \n HFD mice")
```



Export filtered and normalized RNAseq data

```
saveRDS(RNAseq, file = 'results/bulkRNAseq_mmus_data_filt_norm.rds')
```

```
sessionInfo()
```

```
## R version 4.0.5 (2021-03-31)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
```

```
## locale:
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
##  [1] grid      parallel  stats4    stats     graphics  grDevices utils
##  [8] datasets  methods   base
##
## other attached packages:
##  [1] ggrepel_0.9.1               org.Mm.eg.db_3.11.4
##  [3] AnnotationDbi_1.50.3        clusterProfiler_3.16.1
##  [5] VennDiagram_1.6.20          futile.logger_1.4.3
##  [7] DESeq2_1.28.1               SummarizedExperiment_1.18.2
##  [9] DelayedArray_0.14.1         matrixStats_0.58.0
## [11] Biobase_2.48.0              GenomicRanges_1.40.0
## [13] GenomeInfoDb_1.24.2         IRanges_2.22.2
## [15] S4Vectors_0.26.1            BiocGenerics_0.34.0
## [17] forcats_0.5.1               stringr_1.4.0
## [19] dplyr_1.0.3                 purrr_0.3.4
## [21] readr_1.4.0                 tidyr_1.2.0
## [23] tibble_3.1.4                ggplot2_3.3.3
## [25] tidyverse_1.3.0
##
## loaded via a namespace (and not attached):
##   [1] readxl_1.3.1           backports_1.2.1        fastmatch_1.1-0
##   [4] plyr_1.8.6             igraph_1.2.6           splines_4.0.5
##   [7] BiocParallel_1.22.0    urltools_1.7.3         digest_0.6.27
##  [10] htmltools_0.5.2        GOSemSim_2.14.2        viridis_0.5.1
##  [13] GO.db_3.11.4           fansi_0.4.2            magrittr_2.0.1
##  [16] memoise_2.0.0          annotate_1.66.0        graphlayouts_0.7.1
##  [19] modelr_0.1.8           enrichplot_1.8.1       prettyunits_1.1.1
##  [22] colorspace_2.0-0       blob_1.2.1             rvest_0.3.6
##  [25] haven_2.3.1            xfun_0.31              crayon_1.4.0
##  [28] RCurl_1.98-1.2         jsonlite_1.7.2         scatterpie_0.1.5
##  [31] genefilter_1.70.0      survival_3.2-7         glue_1.4.2
##  [34] polyclip_1.10-0        gtable_0.3.0           zlibbioc_1.34.0
##  [37] XVector_0.28.0         scales_1.1.1           DOSE_3.14.0
##  [40] futile.options_1.0.1   DBI_1.1.1              Rcpp_1.0.7
##  [43] viridisLite_0.3.0      xtable_1.8-4           progress_1.2.2
##  [46] gridGraphics_0.5-1     bit_4.0.4              europepmc_0.4
##  [49] httr_1.4.2             fgsea_1.14.0           RColorBrewer_1.1-2
##  [52] ellipsis_0.3.2         pkgconfig_2.0.3        XML_3.99-0.5
##  [55] farver_2.0.3           dbplyr_2.0.0           locfit_1.5-9.4
##  [58] utf8_1.1.4             labeling_0.4.2         ggplotify_0.0.5
##  [61] tidyselect_1.1.0       rlang_0.4.10           reshape2_1.4.4
##  [64] munsell_0.5.0          cellranger_1.1.0       tools_4.0.5
##  [67] cachem_1.0.3           downloader_0.4         cli_2.3.0
##  [70] generics_0.1.2         RSQLite_2.2.3          broom_0.7.4
##  [73] ggridges_0.5.3         evaluate_0.14          fastmap_1.1.0
##  [76] yaml_2.2.1             knitr_1.31             bit64_4.0.5
##  [79] fs_1.5.0               tidygraph_1.2.0        ggraph_2.0.4
```

```
##  [82] formatR_1.7            DO.db_2.9              xml2_1.3.2
##  [85] compiler_4.0.5         rstudioapi_0.13        reprex_1.0.0
##  [88] tweenr_1.0.1           geneplotter_1.66.0     stringi_1.5.3
##  [91] highr_0.8              lattice_0.20-41        Matrix_1.3-2
##  [94] vctrs_0.3.8            pillar_1.6.2           lifecycle_0.2.0
##  [97] BiocManager_1.30.10    triebeard_0.3.0        data.table_1.13.6
## [100] cowplot_1.1.1          bitops_1.0-6           qvalue_2.20.0
## [103] R6_2.5.0               gridExtra_2.3          lambda.r_1.2.4
## [106] MASS_7.3-53            assertthat_0.2.1       withr_2.4.1
## [109] GenomeInfoDbData_1.2.3 hms_1.0.0              rmarkdown_2.14
## [112] rvcheck_0.1.8          ggforce_0.3.2          lubridate_1.7.9.2
```